Research Article

Stem Cell & Regenerative Medicine

Primitive Stem Cells in Adult Feline, Canine, Ovine, Caprine, Bovine, and Equine Peripheral Blood

Henry E. Young[1,2*], Frank Lochner[3], Deborah Lochner[3], Douglas Lochner[3], Gypsy F. Black[2], Julie A. Coleman[2], George McCommon[4], and Asa C. Black, Jr[5]

[1]*Dragonfly Foundation for Research and Development, Macon, GA 31210, USA.*

[2]*Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA, 31207, USA.*

[3]*Cougar Creek Farms, Fort Valley, GA 31030, USA.*

[4]*Department of Veterinary Sciences, Fort Valley State University, Fort Valley, GA 31030, USA.*

[5]*Department of Medical Education, Greenville Hospital System, University of South Carolina School of Medicine, Greenville, SC 29605, USA.*

[*]Correspondence:
Henry E. Young, Chief Science Officer, Dragonfly Foundation for Research and Development, 1515 Bass Rd, Suite E (Corporate Office), Macon, GA 31210, Tel: 478-319-1983; Fax: 478-743-0280; E-mail: young.hey1@yahoo.com.

Received: 04 May 2017; **Accepted:** 30 May 2017

Citation: Henry E. Young, Frank Lochner, Deborah Lochner, et al. Primitive Stem Cells in Adult Feline, Canine, Ovine, Caprine, Bovine, and Equine Peripheral Blood. Stem Cells Regen Med. 2017; 1(1) 004: 1-6.

ABSTRACT

Stout et al. [1] reported the presence of primitive endogenous stem cells circulating within adult porcine peripheral blood. The current study was undertaken to determine whether similar primitive stem cells could be isolated from the peripheral blood of adult felines, canines, ovines, caprines, bovines, and equines. Adult cats, dogs, sheep, goats, cows and horses had their blood withdrawn following the guidelines of Fort Valley State University's IACUC. The blood was obtained by venipuncture and processed to obtain primitive stem cells. Cells were counted using 0.4% Trypan blue inclusion/exclusion analysis and stained with carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1) antibody. Totipotent stem cells are both trypan blue and CEA-CAM-1 positive and < 2.0 microns in size; transitional-totipotent/pluripotent stem cells are both trypan blue and CEA-CAM-1 positive & negative and >2.0 to <6.0 microns in size; and pluripotent stem cells are both trypan blue and CEA-CAM-1 negative and 6-8 microns in size. The results show that TSCs, Tr-TSC/PSCs, and PSCs are circulating within the peripheral blood of all species examined. Studies are ongoing to address their functional significance during maintenance and healing.

Keywords

Cats, Dogs, Sheep, Goats, Cows, Horses, Blood, Totipotent Stem Cells, Pluripotent Stem Cells, Healing Cells.

Introduction

The ability to utilize embryonic stem cells (ESCs) in veterinary medicine is hampered by the complexity of the isolation process. In addition, there are also problems with the ability to maintain lineage and genetic stability, control tumor development, and prevent rejection [2]. The use of autologous adult stem cells obtained from the host would avoid many of these issues. Young and colleagues identified three major categories of primitive stem cells located in solid tissues and organs of adult mammals. Commencing with the most undifferentiated cell, these categories are totipotent stem cells (TSCs) [3], pluripotent stem cells (PSCs) [4], and germ layer lineage stem cells (GLSCs) [5]. Any or all of these adult stem cells have the potential for use for replacement therapies in veterinary medicine. However, their isolation from solid tissues, such as adipose tissue [6], for autologous stem cell therapy is painful for the animal and may create wounds that are slow to heal. The use of adult stem cells obtained by venipuncture from the peripheral blood may avoid these difficulties. Koerner et al. [7] obtained fibroblastoid cells from the peripheral blood of equines, but reported that these cells exhibited limited multi-lineage differentiation potential. Recently, Stout et al. [1] reported the presence of endogenous totipotent stem cells circulating within

adult porcine peripheral blood. The current study was undertaken to determine whether similar primitive stem cells could be isolated from adult feline, canine, ovine, caprine, bovine, and equine peripheral blood. If these cells were present, they could possibly be used in veterinary therapies.

Materials and Methods

The use of animals in this study complied with the guidelines of Fort Valley State University Institutional Animal Care and Use Committee. Their use also complied with the criteria of the National Research Council for the humane care of laboratory animals as outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (National Academy Press, 1996).

Tissue Harvest

Feline, canine, ovine, caprine, bovine, and equine blood was obtained by venipuncture following standard acceptable veterinary practice. Four ml of blood were withdrawn using sterile procedure and placed into tubes containing a 15% EDTA solution. The tubes were inverted three to four times to mix the blood with the EDTA and then refrigerated at 4°C for 48 to 96 hours.

Stem Cell Isolation

After 48 to 96 hours of gravity separation, the blood had separated into a floating plasma fraction and a sedimented cellular fraction. The cellular fraction contained hematopoietic stem cells, red blood cells, white blood cells, and most mesodermal stem cells [1,7-9]. The plasma fraction was withdrawn using a sterile pipette, placed in a second sterile tube and refrigerated at 4°C.

Stem Cell Counting

Totipotent stem cells (TSCs) are Trypan blue positive and < 2.0 microns in size [3,10]. Transitional-totipotent stem cell/pluripotent stem cells (Tr-TSC/PSCs) display a peripheral rim that stains with Trypan blue and a central core that does not. They are >2.0 to <6.0 microns in size [10]. Pluripotent stem cells (PSCs) do not stain with Trypan blue and are 6.0 to 8.0 microns in size [4,10].

Fifteen microliters of the plasma fraction (n=6) from each animal was mixed with 15 microliters of sterile-filtered 0.4% w/v Trypan blue (Kodak, Rochester, NY) in Dulbecco's Phosphate Buffered Saline (DPBS, Invitrogen, GIBCO, Grand Island, NY), pH 7.4. The resulting solution was placed onto a hemocytometer and the isolated cells counted. All cells within the nine large boxes of a standard hemocytometer were counted and then averaged for the number of cells per large box. The formula to determine final cell number per ml was [(((average number)/5)/5) x 0.25) x 2] = cells x 10^6 cells per ml. Final calculations were based on number of cells per ml for whole blood. Ten separate individuals counted the cells for this study. Table 1 denotes the average from 10 counters for each sample +/- Standard Deviation.

Stem Cell Identification

Cells within the plasma fraction were stained with an antibody to carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1) to identify the particular cell types present in the plasma fraction. Totipotent stem cells exhibit staining for CEA-CAM-1 antibody. Transitional-totipotent stem cell/pluripotent stem cells exhibit a peripheral rim that stains with CEA-CAM-1 and a central area that does not stain with the antibody. Pluripotent stem cells do not stain with the CEA-CAM-1 antibody [3,4,10].

In brief, the plasma fraction was placed within 15-ml polypropylene tubes (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) and mixed with an equal volume of ELICA fixative, which consisted of aqueous 0.4% v/v glutaraldehyde, 2% w/v paraformaldehyde, and 1% w/v glucose, Ph 7.4, with an osmolality 1.0. The tissue was fixed for 2 weeks and then rinsed with DPBS [10] at ambient temperature from 5 minutes to 7 days. The plasma / cell / fixative mixture was centrifuged at 2,000 x g. The resultant supernatant was decanted into bleach. The cell pellet was re-suspended and mixed with 14 ml of DPBS pH 7.4 at ambient temperature to wash the fixative from the cells. The cells were centrifuged at 2,000 x g, the resultant supernatant decanted into bleach, and the cells re-suspended. This washing process was repeated a second time to ensure the removal of the fixative from the cells. The cells then underwent the same immunocytochemical staining sequence used for tissue sections.

Immunocytochemistry

Fixed cells were stained using the Enzyme-Linked Immuno-Culture Assay (ELICA) construct [11] for carcinoembryonic antigen cell adhesion molecule-1 epitope (clone 5.4) (CEA-CAM-1) [10]. The fixed cells from the plasma fraction were incubated with 95% ethanol and then washed with 14 ml of DPBS. The cells were incubated with 5 ml of 5.0% (w/v) sodium azide (Sigma, St. Louis, MO) in DPBS for 60 minutes. They were washed with DPBS and incubated with 5 ml of 30% hydrogen peroxide (Sigma, St. Louis, MO) for 60 minutes to irreversibly inhibit endogenous peroxidases [10-12]. The cells were rinsed with DPBS, and incubated for 60 minutes with blocking agent (Vecstain ABC Reagent Kit, Vector Laboratories Inc., Burlingame, CA) in DPBS [10,11]. The blocking agent was removed by centrifugation, and the supernatant decanted. The cells were re-suspended and washed with DPBS. The cells were incubated with primary antibody for 60 minutes. The primary antibody consisted of 0.005% (v/v) CEA-CAM-1, clone 5.4 in DPBS [3,10]. The primary antibody was removed by centrifugation and the supernatant decanted. The cells were re-suspended and washed with DPBS. The cells were incubated with secondary antibody for 60 minutes. The secondary antibody consisted of 0.005% (v/v) biotinylated affinity purified, rat adsorbed anti-mouse immunoglobulin G (H + L) (BA-2001, Vector Laboratories) in DPBS [4,10]. The secondary antibody was removed by centrifugation and the supernatant decanted. The cells were re-suspended and washed with DPBS. The cells were incubated with avidin-HRP for 60 minutes. The avidin-HRP consisted of 10 ml of 0.1% (v/v) Tween-20 (ChemPure, Curtain Matheson Scientific, Houston, TX) containing 2 drops reagent-A and 2 drops reagent-B (Peroxidase Standard PK-4000 Vectastain ABC Reagent Kit, Vector Laboratories) in DPBS [4,10]. The avidin-

HRP was removed by centrifugation and the supernatant decanted. The cells were re-suspended and washed with DPBS. The cells were incubated with AEC substrate (Sigma) for 60 minutes [4,10]. The AEC substrate was prepared as directed by the manufacturer. The substrate solution was removed by centrifugation and the supernatant decanted. The cells were re-suspended, and washed with DPBS. Fifteen microliters of re-suspended cells were placed on a hemocytometer and the number of cells counted.

Visual Analysis

Stained cells were visualized using a Nikon TMS phase contrast microscope with bright field microscopy at 40x, 100x, and 200x. Photographs were taken with a Nikon CoolPix 995 digital camera.

Results

Three cell types were visualized with Trypan blue staining in feline (Figure 1A), canine (Figure 1C), ovine (Figure 2A), caprine (Figure 2C), bovine (Figure 3A), and equine (Figure 3C) peripheral blood. Numerous, very small spherical structures that stained completely with the Trypan blue dye were designated as TSCs.

Slightly larger cells with a stained peripheral rim and a clear central area were designated as Tr-TSC/PSCs. The third category of cells was larger than the other cell types and did not stain with Trypan blue dye. They appeared as white (glowing) spheres on a blue background of Trypan blue dye. These cells were designated as PSCs.

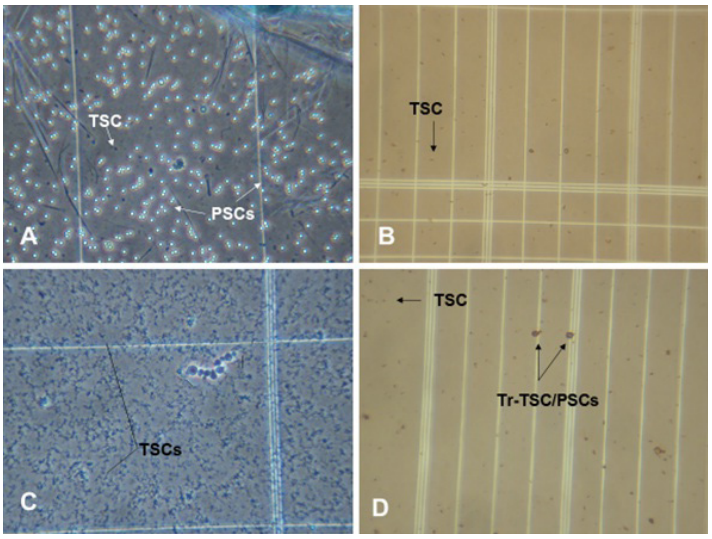

Figure 1: Feline (A,B) and Canine (C,D) stained plasma fractions.

A: Feline plasma fraction stained with 0.4% Trypan blue. TSCs are small dark round circles and PSCs have glowing white nuclei, 100x mag.
B: Feline plasma fraction stained with CEA-CAM-1. TSCs are small dark-red round circles, 100x mag.
C: Canine plasma fraction stained with 0.4%Trypan blue. TSCs are small dark round circles and Tr-TSC/PSCs are structures with a dark periphery and a clear center, 100x mag.
D: Canine plasma fraction stained with CEA-CAM-1. TSCs are small dark-red round circles and Tr-TSC/PSCs are structures with a dark-red periphery and a clear center, 100x mag.

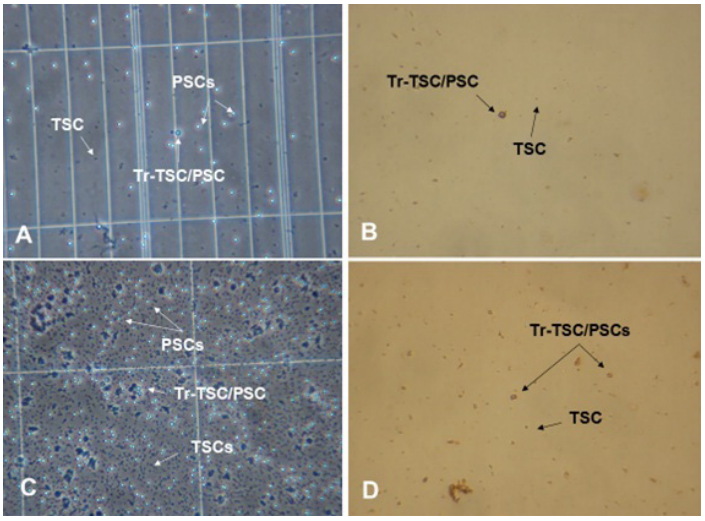

Figure 2: Ovine (Sheep, A,B) and Caprine (Goat, C,D) stained plasma fractions.

A: Ovine plasma fraction stained with 0.4% Trypan blue. TSCs are small dark round circles, Tr-TSC/PSC are round with a dark periphery and a clear center, and PSCs are glowing white rounds, 100x mag.
B: Ovine plasma fraction stained with CEA-CAM-1. TSCs are small dark-red round circles, Tr-TSC/PSC have a dark red periphery and a clear center, and PSCs are unstained, 100x mag.
C: Caprine plasma fraction stained with 0.4%Trypan blue. TSCs are small dark round circles, Tr-TSC/PSC are round with a dark periphery and a clear center, and PSCs are glowing white rounds, 100x mag.
D: Caprine plasma fraction stained with CEA-CAM-1. TSCs are small dark-red round circles, Tr-TSC/PSC have a dark red periphery and a clear center, and PSCs are unstained, 100x mag.

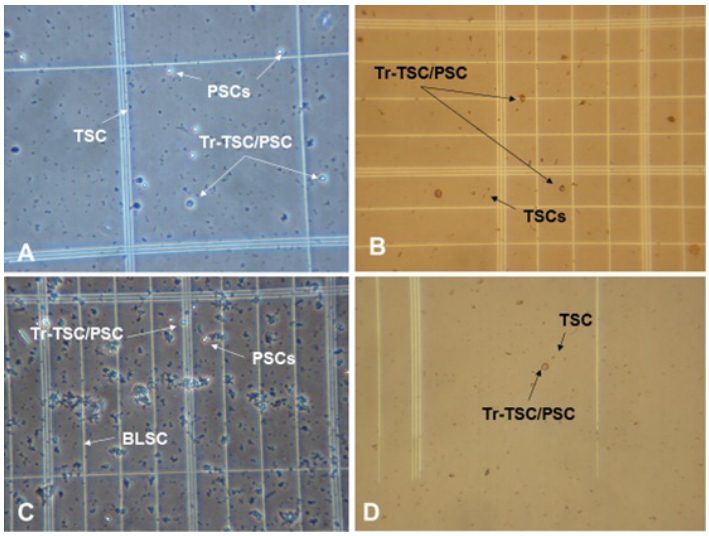

Figure 3: Bovine (A,B) and Equine (C,D) stained plasma fractions.

A: Bovine plasma fraction stained with 0.4% Trypan blue. TSCs are small dark round circles, Tr-TSC/PSC are round with a dark periphery and a clear center, and PSCs are glowing white rounds, 100x mag.
B: Bovine plasma fraction stained with CEA-CAM-1. TSCs are small dark-red round circles, Tr-TSC/PSC have a dark red periphery and a clear center, and PSCs are unstained, 100x mag.
C: Equine plasma fraction stained with 0.4%Trypan blue. TSCs are small dark round circles, Tr-TSC/PSC are round with a dark periphery and a

clear center, and PSCs are glowing white rounds, 100x mag.
D: Equine plasma fraction stained with CEA-CAM-1. TSCs are small dark-red round circles, Tr-TSC/PSC have a dark red periphery and a clear center, and PSCs are unstained, 100x mag.

Staining for CEA-CAM-1 was examined in feline (Figure 1B), canine (Figure 1D), ovine (Figure 2B), caprine (Figure 2D), bovine (Figure 3B), and equine (Figure 3D) peripheral blood. As with the Trypan blue staining, the entities designated as TSCs were very small circular structures that stained with the CEA-CAM-1 antibody. Larger circular structures with a rim of staining for CEA-CAM-1 and a clear center were designated as Tr-TSC/PSCs. PSCs were devoid of CEA-CAM-1 staining.

The counting results obtained with Trypan blue staining clearly demonstrated the existence of totipotent stem cells and pluripotent stem cells in cats, dogs, sheep, goats, cows, and horses and that there were variations in the numbers and types of these stem cells in the six species examined (Table 1).

Species	TSC	+/- Std Dev	Tr-TSC/PSCs	+/- Std Dev
Feline	253	200	120	134
Canine	500	130		
Ovine	125	222	100	
Caprine	125	150	145	163
Bovine	175	150	80	135
Equine	151	127		

Table 1: Average Number of Circulating Stem Cells in Adult Animals. X 10^6 Cells per ml of Blood.

Discussion

Young and colleagues [3,8,13,14] have described five main categories of stem cells involved in the healing of body tissues and organs. These categories are based on the size of the cell, the ability to stain with Trypan blue, the identity of cell surface markers, optimal cryopreservation temperatures, doubling times, growth at confluence, capabilities for self-renewal, differentiation potentials and unidirectional developmental lineage patterns. These five major categories of adult stem cells are totipotent stem cells (TSCs), transitional-totipotent stem cell/pluripotent stem cells (Tr-TSC/PSCs), pluripotent stem cells (PSCs), transitional-pluripotent stem cell/germ layer lineage stem cells (Tr-PSC/GLSCs), and the germ layer lineage stem cells, i.e., ectodermal stem cells (EctoSCs), mesodermal stem cells (MesoSCs), and endodermal stem cells (EndoSCs). The TSCs are less than 2 microns in size, and express carcinoembryonic antigen (CD66e/CEA-CAM-1) on their cell surface. They have the capability to form any somatic cell in the body, as well as forming the germ cells such as spermatogonia. Therefore, TSCs are designated as adult-derived totipotent stem cells.

The Tr-TSC/PSCs are >2 to <6 microns in size. These transitional stem cells express the stem cell surface markers characteristic of both TSCs and PSCs, i.e., carcinoembryonic antigen (CD66e/CEA-CAM-1), stage specific embryonic antigen (SSEA), and neutral endopeptidase (CD10). Transitional-TSC/PSCs can form any somatic cell type in the body, but do not form the germ cells. They are therefore designated as pluripotent stem cells.

The PSCs are 6-8 microns in size. These stem cells express the cell surface markers stage specific embryonic antigen (SSEA) and neutral endopeptidase (CD10). PSCs can form any somatic cell type in the body, but do not form the germ cells. They are thus designated as pluripotent stem cells.

The Tr-PSC/GLSCs are 8-10 microns in size. The Tr-PSC/GLSCs express cell surface markers characteristic of both PSCs and GLSCs, i.e., stage specific embryonic antigen (SSEA), neutral endopeptidase (CD10), CD90, and Thy-1. Like the Tr-TSC/PSCs and PSCs, Tr-PSC/GLSCs will form all somatic cells of the body, but will not form the germ cells. Therefore, they are pluripotent.

Germ layer lineage stem cells are 10-20 microns in size. There are three separate categories of GLSCs based on their respective germ layer of origin, i.e., ectodermal, mesodermal, or endodermal. However, all three types of GLSC express the cell surface markers CD90, Thy-1, and MHC-I. The GLSCs will form cells belonging only to the appropriate germ layer from which they originally arose. For example, ectodermal germ layer lineage stem cells (EctoSCs) can form cells of both surface neural ectoderm and surface ectoderm origin, i.e., neurons, macroglial cells, ganglion cells, Schwann cells, melanocytes, sensory nerve endings, motor nerve endings, and epidermal cells, hair follicles, sweat glands, etc. Mesodermal germ layer lineage stem cells (MesoSCs) can form cell types from somatic mesoderm, intermediate mesoderm, and (somatic and splanchnic) lateral plate mesoderm origin, i.e., three types of muscle, two types of fat, five types of cartilage, two types of bone, multiple types of connective tissue stroma, scar tissue, multiple types of endothelial cells, vasculature, hematopoietic cells, tissues within the genitourinary system (except the germ cells, sperm or ova), mesothelium, spleen, adrenal cortex, etc. Endodermal germ layer lineage stem cells (EndoSCs) can form gastrointestinal lining cells, cystic lining cells, hepatocytes, canalicular cells, biliary cells, pancreatic exocrine acinar cells and ducts, pancreatic endocrine alpha cells, beta cells, delta cells, thymus, thyroid, parathyroid, etc.

Adult stem cells are being used experimentally in multiple animal species to treat a variety of conditions [15-20]. Mesenchymal stem cells have been the focus of many studies. These cells have been defined as somatic cell populations found most commonly in bone marrow [21,22]. However, they are also found in various organs, such as adipose tissue [6] and the connective tissue stroma associated with skeletal muscle [10]. Questions have persisted regarding whether these mesenchymal stem cells (MSC's) are legitimate multipotent stem cells or whether they are simply collections of cell- and tissue-committed progenitor cells derived from connective tissue [23].

Reports of canine stem cell studies include both embryonic and adult stem cells. Canine stem cells have been isolated from

numerous anatomical locations in fetal, neonatal and adult dogs. Stem cells have been studied in a variety of canine animal and human disease models for both basic and clinical research [24-43]. For example, Urbanek et al. [15] showed the repair of damaged cardiac tissue using activated growth factors with adult stems cells in a canine model of heart disease.

Equine stem cells have been used in the treatment of injuries to tendons and ligaments [16-18]. Equine autologous mesenchymal stem cells have also been used to increase the rate of healing in a surgically repaired defect of the equine soft palate. Since many equine injuries involve the musculoskeletal system, the use of autologous stem cells obtained from peripheral blood may be beneficial in the treatment of these conditions.

Because of the use of cats in medical research, feline MSCs derived from bone marrow have been characterized. The marrow cells were studied in order to determine their suitability for use in therapy in humans [44]. Studies are currently underway to determine if the transfer of MSCs is of value in the treatment of chronic renal disease in cats [45]. In a similar study, Arriero et al. [20] investigated the ability of skeletal muscle stem cells to differentiate into endothelial cells that improved kidney function after ischemia, a therapy that may be applicable to humans. Small, proliferative ventricular myocytes have been identified in adolescent feline hearts. These cells have immature physiological properties indistinguishable from cardiac stem/progenitor cells. They have been shown to contribute to the hyperplastic growth of the adolescent heart [46].

The results of this project confirm that identifiable adult totipotent stem cells (TSCs) and pluripotent stem cells (Tr-TSC/PSCs, and PSCs) can be isolated from adult feline, canine, ovine, caprine, bovine, and equine peripheral blood. The use of peripheral blood provides a simpler extraction method than current methods of obtaining adult stem cells from adipose tissue or bone marrow extracts. The presence of TSCs and PSCs in all species makes possible the development of treatments with species-specific autologous totipotent and pluripotent stem cells.

The ability to harvest TSCs and PSCs by the collection of peripheral blood rather than the use of more invasive techniques greatly simplifies the acquisition of totipotent stem cells and pluripotent stem cells. The procedure has the potential for making therapeutic advances possible in tissue engineering in all species studied.

Based on these findings, we postulate that totipotent stem cells and pluripotent stem cells can be found in all adult mammalian species. However, further research is necessary to support this hypothesis. We are currently pursuing studies on possible uses for totipotent stem cells and pluripotent stem cells derived from peripheral blood.

Acknowledgment

The authors would like thank M Carriero, O Samples, P Jones, S Fortson, C Alena, V Krishna, L McGill, and FP Boyer III for their technical assistance. The authors would like to thank D Hixson, Department of Internal Medicine, Brown University, Providence, RI, for the kind gift of the CEA-CAM-1 antibody. These studies were funded in part by MedCen Foundation, Rubye Ryle Smith Charitable Trust, and L.M. & H.O. Young Estate Trust.

References

1. Stout CL, Ashley DW, Morgan JH 3rd, et al. Primitive stem cells residing in the skeletal muscle of adult pigs are mobilized into the peripheral blood after trauma. Am Surg. 2007; 73: 1106-1110.
2. Hayes B. Derivation, characterization, and in vitro differentiation of canine embryonic stem cells. Stem Cells. 2007.
3. Young HE, Black Jr AC. Adult-derived stem cells. Minerva Biotechnologica 17:55-63, 2005.
4. Young HE, Duplaa C, Yost MJ, et al. Clonogenic analysis reveals reserve stem cells in postnatal mammals. II. Pluripotent epiblastic-like stem cells. Anat Rec A Discov Mol Cell Evol Biol. 2004; 277: 178-203.
5. Young HE, Duplaa C, Young TM, et al. Clonogenic analysis reveals reserve stem cells in postnatal mammals: I. Pluripotent mesenchymal stem cells. Anat Rec 2001; 263: 350-360.
6. Parker AM, Katz AJ. Adipose-derived stem cells for the regeneration of damaged tissues. Expert Opin Biol Ther. 2006; 6: 567-578.
7. Koerner J, Nesic D, Romero JD, et al. Equine peripheral blood-derived progenitors in comparison to bone marrow-derived mesenchymal stem cells. Stem Cells 2006; 24: 1613-1619.
8. Young HE, Black AC Jr. Adult stem cells. Anat Rec A Discov Mol Cell Evol Biol. 2004; 276: 75-102.
9. McCommon GW. Primitive adult-derived stem cells are present in the blood of adult equines and can be increased in number with moderate exercise or ingestion of a cyanobacter, Aphanizomenon flos-aquae. Autocoids. 2013; 2: 103.
10. Young HE. Location and characterization of totipotent stem cells and pluripotent stem cells in the skeletal muscle of the adult rat. Stem Cells Regen Med. 2017; 1-17.
11. Young HE. Enzyme-linked immuno-culture assay. J of Tiss Cult Meth. 1992; 14: 31-36.
12. Henson NL, Heaton ML, Holland BH, et al. Karyotypic analysis of adult pluripotent stem cells. Histol Histopathol. 2005; 20: 769-784.
13. Young HE, Steele TA, Bray RA, et al. Human pluripotent and progenitor cells display cell surface cluster differentiation markers CD10, CD13, CD56, and MHC class-I. Proc Soc Exp Biol Med. 1999; 221: 63-71.
14. Young HE, Steele TA, Bray RA, et al. Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. Anat Rec 2001; 264: 51-62.
15. Urbanek K, Torella D, Sheikh F, et al. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proc Natl Acad Sci U S A 2005; 102: 8692-8697.
16. Young RG, Butler DL, Weber W, et al. Use of mesenchymal

stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res 1998; 16: 406-413.
17. Herthel DJ. Enhanced suspensory ligament healing in 100 horses by stem cell and other bone marrow components. AAEP Proceedings. 2001; 47: 319-321.
18. Smith RK, Zunino L, Webbon PM, et al. The distribution of cartilage oligomeric matrix protein (COMP) in tendon and its variation with tendon site, age and load. Matrix Biol. 1997; 16: 255-271.
19. Carstanjen B. Successful engraftment of cultured autologous mesenchymal stem cells in a surgically repaired soft palate defect in an adult horse. Can J Vet Res. 2006; 70: 143–147.
20. Arriero M. Adult skeletal muscle stem cells differentiate into endothelial lineage and ameliorate renal dysfunction after acute ischemia. Am J Physiol Renal Physiol. 2004; 287: 621-627.
21. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991; 9: 641-650.
22. Caplan AI. Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. Tissue Eng. 2005; 11: 1198-1211.
23. Phinney DG. Building a consensus regarding the nature and origin of mesenchymal stem cells. J Cell Biochem Suppl. 2002; 38: 7-12.
24. Niemeyer GP, Hudson J, Bridgman R, et al. Isolation and characterization of canine hematopoietic progenitor cells. Exp Hematol. 2001; 29: 686-693.
25. Hatoya S, Torii R, Kondo Y, et al. Isolation and characterization of embryonic stem-like cells from canine blastocysts. Mol Reprod Dev. 2006; 73: 298-305.
26. Bruno B, Goerner MA, Nash RA, et al. Purified canine CD34+Lin- marrow cells transduced with retroviral vectors give rise to long-term multi-lineage hematopoiesis. Biol Blood Marrow Transplant. 2001; 7: 543-551.
27. Hahn J. Immunological Characterization of Canine Hematopoietic Progenitor Cells. Ann Hematol. 1991; 63: 223-226.
28. Csaki C. Chondrogenesis, osteogenesis and adipogenesis of canine mesenchymal stem cells: a biochemical, morphological and ultrastructural study. Histochem Cell Biol. 2007; 128: 507-520.
29. Perin EC. Comparison of Intracoronary and Transendocardial Delivery of allogeneic Mesenchymal Cells in a Canine Model of Acute Myocardial Infarction. J Mol Cell Cardiol. 2007.
30. Wu H, Riha GM, Yang H, et al. Differentiation and proliferation of endothelial progenitor cells from canine peripheral blood mononuclear cells. J Surg Res. 2005; 126: 193-198.
31. Wu Y, Zhang J, Gu Y, et al. Expansion of canine bone marrow-derived endothelial progenitor cells and dynamic observation. Ann Vasc Surg. 2006; 20: 387-394.
32. Stewart DA, Bolin RB, Cheney BA, et al. Characteristics of lympho-myelopoietic stem cells isolated from canine peripheral blood. Adv Exp Med Biol. 1984; 172: 33-48.
33. Debelak-Fehir KM, Catchatourian R, Epstein RB Hemopoietic colony forming units in fresh and cryopreserved peripheral blood cells of canines and man. Exp Hematol. 1975; 3: 109-116.
34. Wijewardana V, Sugiura K, Shigeyama N, et al. Isolation and characterization of hematopoietic progenitor cells in canine bone marrow. Vet Immunol Immunopathol. 2007; 115: 230-238.
35. Lim JH, Byeon YE, Ryu HH, et al. Transplantation of canine umbilical cord blood-derived mesenchymal stem cells in experimentally induced spinal cord injured dogs. J Vet Sci. 2007; 8: 275-282.
36. Sampaolesi M, Blot S, D'Antona G, et al. Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. Nature. 2006; 444: 574-579.
37. Kadiyala S, Young RG, Thiede MA, et al. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplant. 1997; 6: 125-134.
38. Kreja L, Weinsheimer W, Nothdurft W. In vitro studies on the radiosensitivity of multipotent hemopoietic progenitors in canine bone marrow. Exp Hematol. 1991; 19: 755-758.
39. Kadiyala S, Young RG, Thiede MA, et al. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplant. 1997; 6: 125-134.
40. Lutty GA, Merges C, Grebe R, et al. Canine retinal angioblasts are multipotent. Exp Eye Res. 2006; 83: 183-193.
41. Fliedner TM. Blood stem cell transplantation: from preclinical to clinical models. Stem Cells. 1995; 13 Suppl 3: 1-12.
42. Milward EA. Isolation and Transplantation of Multipotential Populations of Epidermal Growth factor-responsive, Neural Progenitor Cells from the Canine Brain. J Neurosci Res. 1997; 50: 862-871.
43. de Revel T, Appelbaum FR, Storb R, et al. Effects of granulocyte colony-stimulating factor and stem cell factor, alone and in combination, on the mobilization of peripheral blood cells that engraft lethally irradiated dogs. Blood. 1994; 83: 3795-3799.
44. Martin DR, Cox NR, Hathcock TL, et al. Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. Exp Hematol. 2002; 30: 879-886.
45. Dow S. Mesenchymal Stem Cell Transfer for treatment of Chronic Renal Disease in Cats. Grant from The Winn Feline Foundation. 2007.
46. Chen X. Adolescent Feline Heart Contains a Population of Small Proliferative Ventricular Myocytes with Immature Physiological Properties. Circ Res. 2007; 100: 536-544.